

CLAIMS

What is claimed is:

- 5 1. A method for producing mutagenized polynucleotides from a target sequence, comprising:
- (a) forming a sample comprising
- (i) a target sequence including a section to be mutagenized,
- (ii) a first primer including a sequence complementary to a 3'
- 10 sequence of a sense strand of the section of the target sequence,
- (iii) a second primer including a sequence complementary to a 3' sequence of an antisense strand of the section of the target sequence, and
- (iv) at least one oligonucleotide;
- 15 (b) performing at least one cycle of primer extension amplification on the sample in the presence of at least one polymerase such that the oligonucleotide anneals to the section of either the sense or antisense strand of the target sequence to form an imperfect double-stranded sequence and is extended by the polymerase; and
- 20 (c) performing additional cycles of primer extension amplification on the sample to form a mutagenized double-stranded polynucleotide comprising sequences of the first and second primers and the sequence of the oligonucleotide extended in step (b).
- 25 2. The method according to claim 1 wherein the at least one oligonucleotide includes a portion which is complementary to the target sequence and a portion which is not complementary to the target sequence relative to where the oligonucleotide anneals to the target sequence during primer extension amplification, the portion which is not
- 30 complementary to the target sequence being unknown at the time of primer extension amplification.

3. The method according to claim 1, wherein the at least one oligonucleotide has a sequence which is unknown at the time of primer extension amplification.

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4. The method according to claim 1, wherein a portion of the target sequence to which the at least one oligonucleotide anneals during primer extension amplification is unknown at the time of primer extension amplification.

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5. The method according to claim 1, wherein the target sequence has a sequence which at least partially unknown at the time of primer extension amplification.

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6. The method according to claim 1, wherein the target sequence has a sequence which is the CDR of an antibody.

7. The method according to claim 1, wherein the target sequence has a sequence encoding a single-chain antibody.

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8. The method according to claim 1, wherein the first and second primers include at least one restriction site.

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9. The method according to claim 1, wherein one of the first and second primers includes an ATG or an GTA sequence and the other primer includes a sequence encoding a translation stop codon.

10. The method according to claim 1, wherein the lengths of the first and second primers is between 10 and 80 nucleotides.

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11. The method according to claim 1, wherein the at least one oligonucleotide has a length between 10 and 80 nucleotides.

5 12. The method according to claim 1, wherein the at least one oligonucleotide has a length between 10 and 50 nucleotides.

13. The method according to claim 1, wherein the at least one oligonucleotide has a length between 15 and 30 nucleotides.

10 14. The method according to claim 1, wherein the at least one oligonucleotide includes 1-5 inosine residues at the 3' end.

15 15. The method according to claim 1, wherein the at least one oligonucleotide includes 2-4 inosine residues at the 3' end.

16. The method according to claim 1, wherein at least a portion of the multiple cycles of primer extension polymerase amplification is performed such that extension by the polymerase is at least partially performed at a temperature below 70°C for at least 30 sec.

20 17. The method according to claim 1, wherein at least a portion of the multiple cycles of primer extension polymerase amplification is performed such that extension by the polymerase is at least partially performed at a temperature below 60°C for at least 30 sec.

25 18. The method according to claim 1, wherein at least a portion of the multiple cycles of primer extension polymerase amplification is performed such that extension by the polymerase is at least partially performed at a temperature below 50°C for at least 30 sec.

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19. The method according to claim 1, wherein at least a portion of the multiple cycles of primer extension polymerase amplification is performed such that extension by the polymerase is at least partially performed by heating the amplification reaction mixture from temperature of between 30°C to 50°C to a temperature between 65°C to 75°C over the course of at least 30 sec.
20. The method according to claim 1, wherein the imperfect double-stranded sequence formed during the at least one cycle of primer extension amplification includes a bulge.
21. The method according to claim 1, wherein the imperfect double-stranded sequence formed during the at least one cycle of primer extension amplification includes a loop.
22. The method according to claim 1, wherein the library of mutagenized polynucleotides formed may include homologs of the target sequence where at least two sequences from the oligonucleotides have been inserted.
23. The method according to claim 1, wherein the mutagenized polynucleotides formed may include homologs of the target sequence where at least two portions of the target sequence have been deleted.
24. The method according to claim 1, wherein the mutagenized polynucleotides includes sequences that have been mutagenized at at least two separate locations relative to the target sequence.
25. A method for producing a library of mutagenized polynucleotides from a target sequence comprising:
forming a sample comprising

(i) a target sequence including a section to be mutagenized,
(ii) a first primer including a sequence complementary to a 3'
sequence of a sense strand of the section of the target sequence,
(iii) a second primer including a sequence complementary to
a 3' sequence of an antisense strand of the section of the target
sequence, and

(iv) a library of oligonucleotides; and
performing multiple cycles of primer extension amplification on
the sample using a polymerase where primer extension is performed
under conditions suitable for the oligonucleotides to anneal to the target
sequence or amplification products thereof to form imperfect double-
stranded sequences and be extended by the polymerase;

wherein a library of mutagenized polynucleotides are produced
as amplification products of the multiple amplification cycles.

26. The method according to claim 25, wherein the oligonucleotides
in the library include a portion which is complementary to the target
sequence and a portion which is not complementary to the target
sequence relative to where the oligonucleotide anneals to the target
sequence during primer extension amplification, the portion which is not
complementary to the target sequence being unknown at the time of
primer extension amplification.

27. The method according to claim 21, wherein the oligonucleotides
in the library have a sequence which is unknown at the time of primer
extension amplification.

28. The method according to claim 25, wherein a portion of the target
sequence to which the oligonucleotides anneal during primer extension
amplification is unknown at the time of primer extension amplification.

29. The method according to claim 25, wherein the target sequence has a sequence which at least partially unknown at the time of primer extension amplification.

5 30. The method according to claim 25, wherein the target sequence has a sequence which is the CDR of an antibody.

31. The method according to claim 25, wherein the target sequence has a sequence encoding a single chain antibody.

10 32. The method according to claim 25, wherein the at least one oligonucleotide has a length between 10 and 80 nucleotides.

15 33. The method according to claim 25, wherein the at least one oligonucleotide has a length between 10 and 50 nucleotides.

34. The method according to claim 25, wherein the at least one oligonucleotide includes 1-5 inosine residues at the 3' end.

20 35. The method according to claim 25, wherein the at least one oligonucleotide includes 2-4 inosine residues at the 3' end.

25 36. The method according to claim 25, wherein at least a portion of the multiple cycles of primer extension polymerase amplification is performed such that extension by the polymerase is at least partially performed at a temperature below 55°C for at least 30 sec.

30 37. The method according to claim 25, wherein the library of mutagenized polynucleotides formed may include homologs of the target sequence where at least two sequences have been inserted or deleted.

38. The method according to claim 25, wherein the mutagenized polynucleotides includes sequences that have been mutagenized at at least two separate locations relative to the target sequence.

39. A method for producing a library of mutagenized polynucleotides from a double-stranded target sequence, comprising:

(a) forming a sample comprising

(i) a target sequence having sense and antisense strands

and including a section to be mutagenized,

(ii) a first primer including a sequence complementary to a 3' sequence of the section of the sense strand of the target sequence,

(iii) a second primer including a sequence complementary to a 3' sequence of the section of the antisense strand of the target sequence, and

(iv) a library of oligonucleotides;

(b) performing at least one cycle of primer extension amplification on the sample in the presence of at least one polymerase such that at least one of the oligonucleotides anneals to either the sense or antisense strand of the target sequence to form an imperfect double-stranded sequence and is extended by the polymerase; and

(c) performing additional cycles of primer extension amplification on the sample to form a randomly mutagenized double-stranded polynucleotide comprising sequences of the first and second primers and the sequence of the oligonucleotide extended in step (b).

40. The method according to claim 39, wherein the oligonucleotides in the library include a portion which is complementary to the target sequence and a portion which is not complementary to the target sequence relative to where the oligonucleotide anneals to the target sequence during primer extension amplification, the portion which is not

complementary to the target sequence being unknown at the time of primer extension amplification.

41. The method according to claim 39, wherein the oligonucleotides in the library have a sequence which is unknown at the time of primer extension amplification.

42. The method according to claim 39, wherein a portion of the target sequence to which the oligonucleotides anneal during primer extension amplification is unknown at the time of primer extension amplification.

43. The method according to claim 39, wherein the target sequence has a sequence which at least partially unknown at the time of primer extension amplification.

44. The method according to claim 39, wherein the at least one oligonucleotide has a length between 10 and 80 nucleotides.

45. The method according to claim 39, wherein the at least one oligonucleotide has a length between 10 and 50 nucleotides.

47. The method according to claim 39, wherein the at least one oligonucleotide includes 1-5 inosine residues at the 3' end.

48. The method according to claim 39, wherein the at least one oligonucleotide includes 2-4 inosine residues at the 3' end.

49. The method according to claim 39, wherein at least a portion of the multiple cycles of primer extension polymerase amplification is performed such that extension by the polymerase is at least partially performed at a temperature below 55°C for at least 30 sec.

